Available online at www.sciencedirect.com
ScienceDirect
Aquacultural Engineering 38 (2008) 79–92
aquacultural engineering
www.elsevier.com/locate/aqua-online

# Comparing denitrification rates and carbon sources in commercial scale upflow denitrification biological filters in aquaculture

H.J. Hamlin [a,*], J.T. Michaels [a], C.M. Beaulaton [a], W.F. Graham [a], W. Dutt [a], P. Steinbach [b], T.M. Losordo [c], K.K. Schrader [d], K.L. Main [a]

[a] *Mote Marine Laboratory, Center for Aquaculture Research and Development, 1600 Ken Thompson Pkwy, Sarasota, FL 34236, USA*
[b] *Neugasse 23d, D67169 Kallstadt, Germany*
[c] *North Carolina State University, Department of Biological & Agricultural Engineering, Raleigh, NC 27695-7625, USA*
[d] *U.S. Department of Agriculture, Agricultural Research Service, Natural Products Utilization Research Unit, National Center for Natural Products Research, Post Office Box 8048, University, MS 38677, USA*

Received 4 June 2007; accepted 15 November 2007

**Abstract**

Aerobic biological filtration systems employing nitrifying bacteria to remediate excess ammonia and nitrite concentrations are common components of recirculating aquaculture systems (RAS). However, significant water exchange may still be necessary to reduce nitrate concentrations to acceptable levels unless denitrification systems are included in the RAS design. This study evaluated the design of a full scale denitrification reactor in a commercial culture RAS application. Four carbon sources were evaluated including methanol, acetic acid, molasses and Cerelose™, a hydrolyzed starch, to determine their applicability under commercial culture conditions and to determine if any of these carbon sources encouraged the production of two common ''off-flavor'' compounds, 2-methyisoborneol (MIB) or geosmin. The denitrification design consisted of a 1.89 $m^3$ covered conical bottom polyethylene tank containing 1.0 $m^3$ media through which water up-flowed at a rate of 10 lpm. A commercial aquaculture system housing 6 metric tonnes of Siberian sturgeon was used to generate nitrate through nitrification in a moving bed biological filter. All four carbon sources were able to effectively reduce nitrate to near zero concentrations from influent concentrations ranging from 11 to 57 mg/l $NO_3$–N, and the maximum daily denitrification rate was 670–680 g nitrogen removed/$m^3$ media/day, regardless of the carbon source. Although nitrite production was not a problem once the reactors achieved a constant effluent nitrate, ammonia production was a significant problem for units fed molasses and to a less extent Cerelose™. Maximum measured ammonia concentrations in the reactor effluents for methanol, vinegar, Cerelose™ and molasses were $1.62 \pm 0.10$, $2.83 \pm 0.17$, $4.55 \pm 0.45$ and $5.25 \pm 1.26$ mg/l $NH_3$–N, respectively. Turbidity production was significantly increased in reactors fed molasses and to a less extent Cerelose™. Concentrations of geosmin and MIB were not significantly increased in any of the denitrification reactors, regardless of carbon source. Because of its very low cost compared to the other sources tested, molasses may be an attractive carbon source for denitrification if issues of ammonia production, turbidity and foaming can be resolved.


*Keywords:* Recirculating aquaculture system; Nitrate; Denitrification; Carbon; Off-flavor

## 1. Introduction

In recent years the aquaculture industry has received considerable criticism due to perceived negative environmental effects from the excessive consumption

* Corresponding author. Present address: 223 Bartram Hall, University of Florida, Gainesville, FL 32611, USA.
Tel.: +1 352 392 1098; fax: +1 352 392 3704.
*E-mail address:* hjhamlin@ufl.edu (H.J. Hamlin).

0144-8609/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaeng.2007.11.003

of water and subsequent release of wastewater. Heightened environmental standards have led, in part, to the concept of sustainable aquaculture, which has received much attention in the last decade, and governmental policies have been established to promote its development and practice (Buschmann et al., 1996; Houte, 2000; Olin, 2001; Harache, 2002; Cranford et al., 2003; Pita et al., 2006). Although specific definitions of sustainable aquaculture are varied (FAO, 1995; Boyd and Tucker, 1998), limited water use is a critical component of any definition and there is a growing demand from consumers for products grown in environmentally responsible systems (Frankic and Hershner, 2003). Numerous efforts are currently underway to develop ''zero discharge'' recirculation systems (Suzuki et al., 2003; Sharrer et al., 2007).

In order to be profitable, however, aquaculture farms first need to be self-sustaining, and growth in aquaculture has led to some interesting paradoxes. In order to be profitable, farmers often feed high protein feeds in great quantity to increase fish growth rates. This leads to significantly more nitrogenous waste (i.e. ammonia, nitrite and nitrate), which may be discharged in large amounts unless it is captured and treated before discharge. Aerobic biological filtration systems employing nitrifying bacteria to reduce concentrations of ammonia and nitrite–nitrogen have become commonplace in freshwater intensive tank-based recirculating aquaculture systems (Timmons et al., 2001; Hall et al., 2002). These technologies are well understood and are decidedly effective at reducing ammonia and nitrite–N concentrations in production systems to acceptable levels (Sharma and Ahlert, 1977). Nitrate–N is the end result of the nitrification process and is removed either by a denitrification process that ideally converts nitrate–N to nitrogen gas or by water exchange.

Denitrification systems that reduce the concentration of nitrate–N are much less common in commercial aquaculture, and the industry has been slow to adopt this technology for several reasons. First and foremost, denitrification systems are challenging to operate and generally costly to build. For flow-through facilities, which have access to large quantities of water at low costs, there is little incentive to adopt this technology. Pond culture systems have little buildup of nitrate–N as denitrification is a natural process taking place at the water/pond bottom interface (Losordo and Westeman, 1994; Gutierrez-Wing and Malone, 2006) and ammonia nitrogen and nitrate are taken up directly by microscopic algae and plants in the pond. Discharge of nitrogen in any form has detrimental effects on the environment, and in the future, more stringent effluent regulations on aquaculture production will place new limits on new and existing production facilities.

If aquaculture is to keep pace with global demand, new production facilities will need to be built, and these new facilities will not have access to the quantities of water that established facilities have had. Additionally, these facilities may not be able to discharge wastewater with excessive concentrations of organic or inorganic nitrogen. Further, nitrate has traditionally been viewed as relatively non-toxic to aquatic species (Russo, 1985; Hrubec, 1996; Jensen, 1996; Van Rijn, 1996), because unlike ammonia or nitrite–N, in which studies have shown significant pathological effects at elevated concentrations, few studies are available detailing the effects of nitrate–N exposure. Evidence from recent studies, however, has shown elevated nitrate concentrations to be a significant concern for a number of commercially relevant aquatic species, demonstrating both lethal and non-lethal effects (Hamlin, 2006; Guillette and Edwards, 2005; Hrubec, 1996). Finally, a universally accepted and readily available concept for the design and operation of a commercial scale denitrification system has not yet been developed and implemented by the aquaculture community (Grguric et al., 2000; Menasveta et al., 2001; Klas et al., 2006; Van Rijn et al., 2006).

The process of nitrate removal converts nitrate to more reduced inorganic nitrogen species, and employs two primary bacterial groups. The first group reduces nitrate to either nitrite or ammonia, and the second group converts nitrate, via nitrite, to dinitrogen gas ($N_2$). The production and accumulation of nitrite from nitrate is often referred to as incomplete denitrification. Elevated nitrite can be of considerable concern as it causes methemoglobinemia, commonly termed brown-blood disease in fish, which reduces the oxygen carrying capacity of the fish's blood (Boyd and Tucker, 1998). Methemoglobinemia can be fatal if the condition is severe. To ensure complete denitrification, an external carbon source is often used that serves as the electron donor and facilitates the denitrification process (Grommen et al., 2006; Van Rijn et al., 2006). Although methanol is the most commonly used amendment, other carbon sources can be used including commercially available starches, sugars and other alcohols (Sperl and Hoare, 1971; Kessreu et al., 2003).

Carbon limiting the denitrification process results in incomplete denitrification and a concomitant accumulation of nitrite. Conversely, an excess of organic electron donors can result in the production of hydrogen sulfide, which can also pose a toxicological threat to the cultured product (Spotte, 1979). Therefore, regulating

carbon additions is critical to properly removing nitrate from the aquatic system through biological denitrification without deleterious effects. Measuring the oxidation reduction potential (ORP) in the denitrification media has been cited as an operationally practical method of ensuring that complete denitrification is occurring while reducing the likelihood of toxic sulfide production (Breck, 1974; Balderston and Sieburth, 1976; Lee et al., 2000). Complete denitrification results in an ORP of $<-200$ mV (Sillén, 1965).

In recirculation systems with limited water exchange, the process of nitrification leads to reductions in alkalinity and a concomitant decline in pH. These reductions are remedied with the routine addition of alkalinity supplements such as sodium bicarbonate. Denitrification results in an increase in alkalinity (Kim and Bae, 2000), and depending on the rate of denitrification and alkalinity of any makeup water, should reduce the expense of alkalinity supplements.

The problem of ''off-flavor'' in the cultured product is an economically significant problem in aquaculture. Microorganisms, such as *Micromonospora* species capable of producing earthy or musty off-flavor compounds can grow in low oxygen or anoxic environments, similar to those present in denitrification environments (Johnston and Cross, 1976). Two of the most well documented compounds implicated in off-flavor are 2-methylisoborneol (MIB) and geosmin (Schrader and Rimando, 2003). System components capable of generating these compounds could threaten the economic viability of the cultured product.

The purpose of this study was to evaluate a design for a commercial scale denitrification system using readily available materials and to evaluate its potential use in commercial aquaculture. In addition, four carbon sources including methanol, acetic acid (vinegar), molasses and Cerelose™, a readily available starch, were examined to determine their performance and applicability under commercial aquaculture conditions and to determine if any of these carbon sources encourage the production of either MIB or geosmin, two ''off-flavor'' compounds that can adversely affect the flavor quality of aquaculture products.

## 2. Materials and methods

### 2.1. Denitrification filter design

The denitrification filter consisted of a 1.89 $m^3$, 122 cm diameter, 170 cm high covered conical bottom (15 angular degree) polyethylene tank (Snyder Industries, part # 589045001, Lincoln, NE) containing 1.0 $m^3$ plastic extruded floating media (AMB™ media, EEC) (Fig. 1). Water was pumped up through the extruded plastic media bed at a flow rate of 10 lpm. The filter media bed was backwashed weekly by fluidization and mixing with air injected through a grid at the bottom of the reactor above the conical bottom. The released solids (mostly bacterial cells) were settled, collected and thickened in the cone and removed through a bottom drain (5 cm diameter). An expanded metal screen in the bottom of the tank above the cone prevented the media from exiting the waste drain during the solids removal process. The units were completely drained during backwashing. Important to this design is the fact that the media filled only a portion of the reactor volume to allow adequate space for mixing during backwashing. The water exited the denitrifying filter reactor at the top of the unit through a perforated PVC pipe covered with plastic mesh. The carbon sources were injected into the culture water inflow (see Fig. 1) with a ceramic piston pump (FMI Fluid metering QG150-Q1CKCW/Q2CKCW, Syosset, NY).

### 2.2. The aquaculture system

A commercial aquaculture system holding 6 metric tonnes of Siberian sturgeon (*Acipenser baeri*) was used to generate nitrate through nitrification in a moving bed biological filter (Fig. 2). Approximately 7570 l/m of recirculating water, from a system of four 70.0 metric tonne fish tanks, flowed by gravity through pipes and an open channel to a rotating 60 μm drum screen filter (PR Aqua Rotofilter Model 4872, Nanaimo, BC, Canada) for solids removal before flowing into the moving bed biofilter containing 25 $m^3$ of aerated extruded plastic media (AMB™ media, EEC). The water then cascaded over an aluminum weir into a degassing area, where it was vigorously aerated. The water was then pulled under a divider wall into a non-aerated chamber where it was oxygenated with pure oxygen gas by two FAS™ hooded paddlewheel oxygenators (FAS™ Turboxygene LR200, Vago di Lavagno, Italy) prior to being pumped by a low-head, high-volume variable speed pump back to the tanks. The denitrifying reactors were located near the rotating drum filter (Fig. 2) and used water processed through the drum screen filter for the denitrification process. Water exiting the denitrification filters drained into the treated flow-stream from the drum screen filter immediately entering the moving bed biofilter. Fish within the system were fed an average of 50 kg of feed (Silver Cup™; 45% protein, 19% lipid) daily, being distributed by automatic feeders every 30 min on a 24 h cycle.

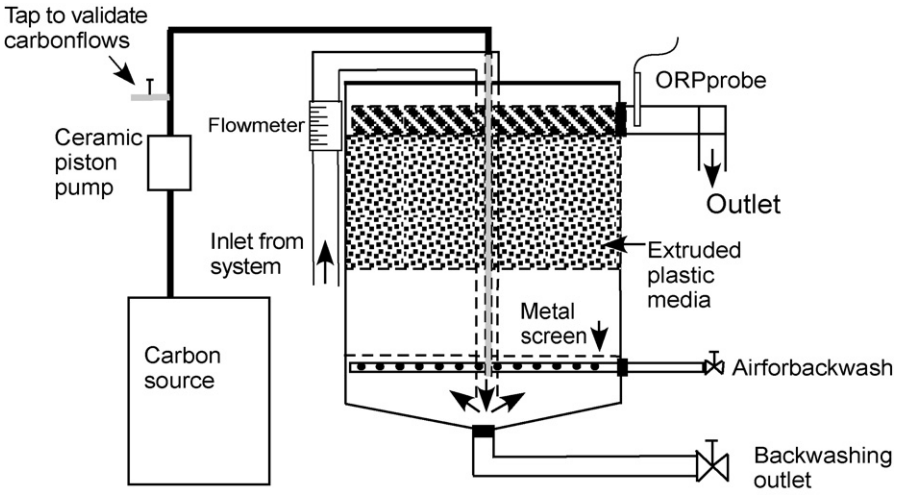


Fig. 1. Schematic diagram (not to scale) of the denitrification assembly for commercial use in aquaculture.

### 2.3. Experimental protocol

Water from the sturgeon culture system was pumped from the drum filter to each of 12 denitrification units at a flow rate of 10 lpm/filter, which produced a mean hydraulic retention time in each 1 $m^3$ bed of approximately 100 min. Three pumps delivered the water to each of 12 denitrification units (1 pump per 4 denitrification units) (Fig. 2) and each denitrification unit had a separate flow meter. Once the nitrate–N concentration in the system averaged 55 mg/l $NO_3$–N, the denitrification filters were engaged and allowed to run until system nitrate–N concentrations dropped to 10 mg/l $NO_3$–N. The filters were then disengaged (turned off and left static) and nitrate concentrations in the fish culture system were allowed to return (via nitrification) to the 55 mg/l $NO_3$–N concentration, at which point the filters were backwashed and again put online. This cycling was repeated three times with the time required for the system nitrate–N concentration to rise to 55 mg/l $NO_3$–N between the experimental cycles being approximately 7–10 days.

The four carbon sources that were evaluated in triplicate included methanol (Simmons Chemical, Sarasota, FL), vinegar (acetic acid, 20% concentration) (Cruzan Int. Inc., Lake Alfred, FL) refinery molasses (#300 standard blackstrap, 70% solids) (U.S. Sugar Corporation, Clewiston, FL) and powdered Cerelose™ (99.5% dextrose) (Corn Products U.S., Westchester, IL). The amount of each carbon source added to the reactors was dictated by the concentration of nitrate in the system, and was dosed based on a grams carbon to grams nitrogen basis (C/N ratio). Methanol, acetic acid, Cerelose™ and molasses were dosed according to a C/N of 2.0, 1.7, 2.5 and 2.5, respectively. The amount of each carbon source pumped to the denitrification filters was adjusted based on carbon content of each source. Startup carbon to nitrogen ratios (g/g) were chosen to

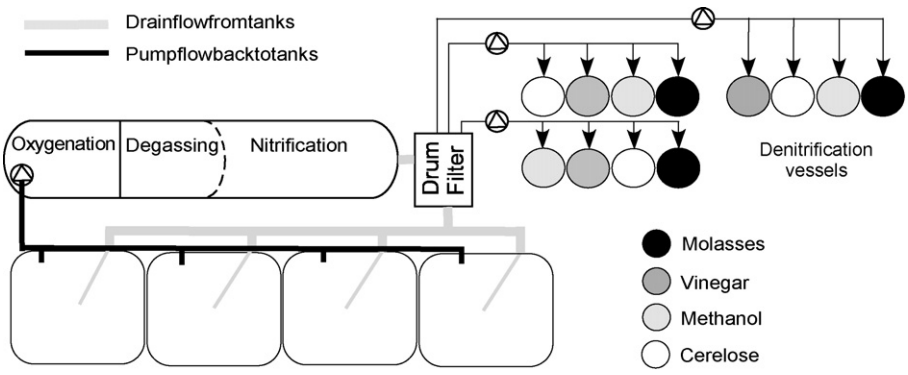


Fig. 2. Schematic diagram (not to scale) of the system configuration and experimental denitrification unit locations.

ensure adequate carbon for complete denitrification of the flow-stream through the denitrifying filter. For methanol a ratio of 2 g C/g N was chosen as this ratio was being successfully used in a similar system in Europe. This gave an equivalent methanol usage of 5.3 g methanol/g N. For acetic acid, a C/N ratio of 1.7 g C/g N was used (Mohseni-Bandpi et al., 1999) resulting in an equivalent acetic acid usage of 4.3 g acetic acid/g N. Because of the uncertainty on the availability of the carbon in the starch (Cerelose™ Dextrose; 99.4% D-glucose, Corn Products Co.) and the molasses (Blackstrap Molasses 300, U.S. Sugar Co.), a conservative approach was taken for the calculation of the startup ratios for these sources. For the starch and the molasses the target C/N ratio was 2.5 (Gomez et al., 2000). The fraction of carbon in simple sugars is 0.4 (40%). Data from Corn Products Co. on BOD (biological oxygen demand) and COD (chemical oxygen demand) of the starch suggested that 71% of this carbon would be available. The starch required to provide an available C/N ratio of 2.5 was 8.8 g starch/g N. A similar calculation was made for molasses using the same factors and taking into account the sugar content of 44%. A molasses to N ratio of 20/1 would be required to achieve an available C/N ratio of 2.5 and these ratios proved effective at facilitating complete denitrification.

Since altering the dosing pumps daily to accommodate declining system nitrate concentrations was not practical, the carbon source dosing rates were adjusted when system concentrations reached 55, 45, 35 and 25 ppm nitrate–N.

For safety in handling, the methanol and acetic acid were diluted with water to 8.7% and 6.0%, respectively. The powdered Cerelose™ was mixed with well water to a concentration of 44% for pumping purposes. The molasses was used as received. A completely randomized design was used when assigning each of the carbon sources to a reactor. In the second cycle, in order to prolong the trial and increase the possibility of methanol reaching a constant effluent nitrate, the Cerelose™, molasses and vinegar fed denitrification units were disengaged at day 20 of operation until it was clear the methanol units were at a relatively constant effluent nitrate. Water and carbon flows were checked daily. Samples from each denitrifier were collected at the outlet of each reactor between 08:00 and 09:00 h daily for chemical analyses. Oxidation–reduction potential (ORP) readings were also documented at the time of collection. The samples were processed immediately after sampling each day.

### 2.4. Chemical analyses

ORP was measured daily with probes (Pinpoint, PH370, American Marine Inc., Ridgefield, CT) placed continuously at the discharge outlet of each denitrification reactor. The probes were cleaned at the beginning and end of trial 1 and at least once daily for trials 2 and 3. The ORP probes were calibrated twice a week for all trials. Total ammonia–N (TAN) concentration was measured using the direct photometric method (Smart 2 Colorimeter, LaMotte Co., Chestertown, MD) with the Nessler reagent method (Greenberg et al., 1992). Nitrite–N concentration was measured photometrically by evaluating the compound formed by diazotization of sulfanilamide and nitrite coupled with *N*-(1-naphthyl)-ethylenediamine (Smart 2 Colorimeter, LaMotte Co.). Total nitrate was measured daily with an ion specific probe (Ion 6, Acorn Series, Oakton Instruments™, Vernon Hills, IL). Nitrate–N concentration was calculated using measured total nitrate concentration divided by 4.4. Initial nitrate–N concentrations were confirmed with an Auto Analyzer™ to ensure accuracy of the results. Turbidity was measured daily using a formazin standard measurement (Smart 2 Colorimeter, LaMotte Co.). The alkalinity concentration was determined by titration (Hach Company™, Loveland, CO) and pH (double junction electrode, Oakton Instruments™) was measured routinely throughout the trials. COD was analyzed using a mercury free digestion with dichromate in the presence of silver salts (Smart 2 Colorimeter, LaMotte Co.).

### 2.5. Analysis of geosmin and MIB levels in water samples

Individual water samples were placed in 20-ml glass scintillation vials, and these vials were covered with foil-lined caps (Fisher Scientific: catalog # 03-337-4). Vials were filled completely so that no air bubbles were present when the vial was capped and then inverted. These samples were maintained at 4 °C until ready for shipping by overnight express service to the USDA, ARS, Natural Products Utilization Research Unit, University, MS, for analysis of geosmin and MIB levels.

The solid-phase micro-extraction (SPME) procedures used to quantify levels of geosmin and MIB in water samples were according to those used by Lloyd et al. (1998) and as modified by Schrader et al. (2003). A CombiPAL autosampler (Leap Technologies, Carrboro, NC) connected to an Agilent 6890 (Agilent, Santa Clara, CA) gas chromatograph–mass spectrometer (GC–MS) were used to analyze samples. Each water

sample was run in triplicate, and mean values were determined for levels of MIB and geosmin. The instrumental detection threshold limit during this study was 1.0 ng/l.

### 2.6. Statistical evaluations

Statistical analyses were performed using StatView for Windows (SAS Institute, Cary, NC, USA). Analysis of variance (ANOVA) of the various parameters was used to compare differences among treatment groups. If significance was determined ($P < 0.05$), Fisher's protected least-significant difference was used to determine differences among treatment means.

## 3. Results and discussion

These results represent the first stage of an intended two-stage study. The purpose of this first study was to determine whether the denitrification design could be used in commercial culture, and determine whether the carbon sources tested were viable options in commercial culture systems. Unfortunately, the commercial production building housing this experiment was destroyed in a fire at the end of this study, negating the possibility of conducting phase two, which would fine tune dosing rates and evaluate the technical performance of single denitrifiers on individual recirculating culture systems.

### 3.1. Theoretical reactions and the production of extracellular material

The consumption of a carbon source used for denitrification is primarily due to three reactions which include the conversion of nitrate to nitrogen gas, the removal of oxygen from the system, and the production of extracellular material by other reactions. If the water entering the reactors is deoxygenated, then there is no consumption of the carbon source by oxygen. In this study, the water input to the reactors was not deoxygenated. Table 1 shows the C/N ratio (mol/mol) that is stoichiometrically required and the daily amount of the carbon source necessary based on daily denitrification. The equations used to calculate the data in Table 1 are shown in Eqs. (1)–(7). For these reactions, Eqs. (1) and (2) are taken from McCarty et al. (1969) and Eqs. (3)–(7) were calculated using the half reactions given in McCarty et al. (1969).

Methanol:

$$O_2 + 0.93CH_3OH + 0.056NO_3^- + 0.056H^+ \rightarrow 0.056C_5H_7O_2N + 0.65CO_2 + 1.69H_2O \quad (1)$$

$$NO_3^- + 1.08CH_3OH + H^+ \rightarrow 0.065C_5H_7O_2N + 0.47N_2 + 0.76CO_2 + 2.44H_2O \quad (2)$$

Acetic acid:

$$O_2 + 0.5CH_3COOH + 0.144NO_3^- + 3.32H^+ \rightarrow 0.144C_5H_7O_2N + 0.716CO_2 + 2.56H_2O \quad (3)$$

$$0.53CH_3COOH + NO_3^- + 3.18H^+ \rightarrow 0.42N_2 + 0.15C_5H_7O_2N + 1.8H_2O + 3.0CO_2 \quad (4)$$

Starch (99.4% glucose):

$$O_2 + 0.332C_6H_{12}O_6 + 0.144NO_3^- + 4H^+ \rightarrow 1.29CO_2 + 0.144C_5H_7O_2N + 1.56H_2O \quad (5)$$

$$0.176C_6H_{12}O_6 + NO_3^- + 2.8H^+ \rightarrow 0.42N + 0.15C_5H_7O_2N + 3.33H_2O + 3.50CO_2 \quad (6)$$

Sucrose is the largest constituent of the sugar in molasses, accounting for 32.5%. Other sugars are fructose 5%, glucose 2.1% and reducing substances as

Table 1
C/N ratio (mol/mol) required based on the stoichiometry and the daily amount of carbon source necessary based in the daily denitrification

| Carbon source | Denitrification rate (g/day nitrate–N) | Required C/N (mol/mol)[a] | Required C (g C/day) | Actual C/N (mol/mol) | Actual C (g C/day) | Cellular material (g/day) |
|---|---|---|---|---|---|---|
| Methanol | 670 | 1.06 | 643 | 2.3 | 1478 | 363 |
| Acetic acid | 670 | 1.05 | 771 | 2.0 | 1592 | 860 |
| Starch[b] | 680 | 1.00 | 694 | 2.9 | 2012 | 872 |
| Molasses[c] | 670 | 1.04 | 628 | 2.9 | 1998 | 900 |

[a] Weighted average of denitrification and oxygen removal C/N.
[b] Glucose.
[c] Sucrose.

dextrose, 10%. Crude protein is also present at 5.7%. For the following reaction, all the sugars in molasses will be assumed to be sucrose.

Molasses:

$$O_2 + 0.0832C_{12}H_{22}O_{11} + 0.144NO_3^- \rightarrow 0.144C_5H_7O_2N + 0.048CO_2 + 0.229H_2O \quad (7)$$

$$0.088C_{12}H_{22}O_{11} + NO_3^- + 1.52H^+ \rightarrow 0.159C_5H_7O_2N + 0.42N_2 + 0.33CO_2 + 3.72H_2O \quad (8)$$

Actual C/N and delivered carbon were more than the theoretical amounts for required denitrification and oxygen removal. This is due to the conservative assumptions made to insure complete denitrification during the experiment. Reductions and fine tuning of carbon dosages will be investigated in future work.

Cellular production calculated in these stoichiometric equations reflects only material produced by denitrification and oxygen reduction processes. The glucose (starch) and sucrose (molasses) are carbohydrates which can encourage the growth of facultative anaerobes with resulting partial fermentation. This growth is at the expense of the true denitrifiers and results in sludge production in the reactors. In this study, an examination of the reactors showed that the highest sludge production was in the molasses units. The order of sludge production from highest to lowest appeared to be molasses > starch > acetic acid > methanol. Other investigators have observed similar results. Cuervo-Lopez et al. (1999) reported that denitrification with glucose resulted in 90% more production of carbohydrate sludge and 190% more protein compared to methanol. Gomez et al. (2000) found similar results with 70% more biofilm growth with sucrose as compared to methanol.

### 3.2. Nitrate

Fig. 3 shows the concentrations of nitrate–N (A) and ORP (B) values as a function of time in the effluent of each of the denitrification reactors fed the various carbon sources. As expected, system nitrate concentrations dropped rapidly with the implementation of the denitrification reactors, despite the low water flows through the units. It took approximately 8 days of operation for the Cerelose™, molasses and vinegar fed reactors to reach a constant effluent nitrate in trial 1, although there was a significant reduction in nitrate

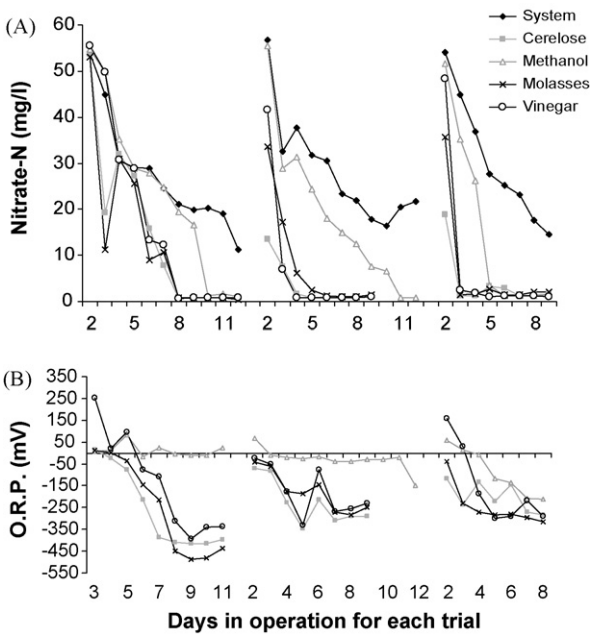


Fig. 3. Nitrate–N (A) and ORP (B) values for commercial denitrification units supplemented with four different carbon sources. The units were engaged when the recirculating system was at 55 mg/l $NO_3$–N and disengaged when the system dropped to 10 mg/l $NO_3$–N. This was repeated for three cycles. The filters remained static in between cycles. On day 20 of operation the Cerelose™, molasses and vinegar fed denitrification units were disengaged to ensure methanol had time to reach a constant effluent nitrate before the end of the trial.

concentration for both Cerelose™ and molasses by only day 3 of operation with outflow concentrations of $19.2 \pm 5.4$ and $11.5 \pm 3.5$ mg/l $NO_3$–N, respectively, with an inflow (system) concentration of 44.7 mg/l $NO_3$–N. The methanol fed reactors took approximately 10 days to reach a constant effluent nitrate. In trial 2, it took only 4 days for Cerelose™ and vinegar to reach a constant effluent nitrate, 5 days for molasses and 11 days for methanol fed reactors. Since we wanted to ensure that all denitrifiers reached a constant effluent nitrate in each trial, we disengaged the Cerelose™, molasses and vinegar fed reactors on day 9 of trial 2, to allow system nitrate concentrations to remain above the 10 mg/l $NO_3$–N threshold long enough for the methanol reactors to reach a constant effluent nitrate, which occurred on day 11 of operation. In trial 3, it took approximately 3 days for the Cerelose™, molasses and vinegar fed units to reach a constant effluent nitrate and the methanol units 5 days. In general, a constant effluent nitrate concentration of $NO_3$–N averaged $0.97 \pm 0.09$ exiting the denitrifying reactors regardless of incoming concentration or carbon source in the tested range of 11–56 mg/l $NO_3$–N.

### 3.3. ORP

Nitrification and denitrification reactions are oxidation/reduction processes whereby electrons are transferred from reducing to oxidizing agents until the reaction has reached an equilibrium. The ORP is the electric potential required to transfer electrons from one compound to another and is often used as a qualitative measure of the state of oxidation of a liquid (Chang et al., 2004). Measured ORP values are related to the changing concentrations of reducing and oxidizing elements and have been used as a qualitative indicator of reaction progress (Kim and Hensley, 1997) with the Nernst equation as follows:

$$E = E^{\circ} + \left(\frac{RT}{nF}\right) \ln\left(\frac{[\text{Oxi}]}{[\text{Red}]}\right) \quad (9)$$

where $E$ is the ORP (mV), $E^{\circ}$ is an ORP standard for the given oxid/red process, $R$ is the gas constant (8.314 J mol$^{-1}$ K$^{-1}$), $T$ is absolute temperature (K), $n$ represents the number of electrons transferred in the reaction, $F$ is the Faraday constant (96500 C mol$^{-1}$), [Oxi] is the oxidation agent concentration and [Red] is the reduction agent concentration. Because the ORP value depends on the ratio between the concentrations of species donating electrons and species accepting electrons, at high nitrate (electron acceptor) concentrations and low electron donor (carbon source) concentrations the ORP value is expected to be higher than a situation in which the nitrate concentration is low and the electron donor is high. In both cases, however, denitrification will occur since the denitrifying bacteria have both an electron acceptor and electron donor, provided oxygen concentrations are close to zero.

It has been stated that complete denitrification takes place at an ORP $> -200$ mV, and that the denitrification process may result in the production of hydrogen sulfide at an ORP $> -400$ mV (Sillén, 1965). Therefore, the ideal range for denitrification is $-200$ to $-400$ mV (Lee et al., 2000). In trial 1, Cerelose™, molasses and vinegar fed units reached a constant effluent nitrate at an ORP value of $-409$, $-451$ and $-311$ mV, respectively (Fig. 3B). The methanol fed units which reached a constant effluent nitrate on day 9 of the 12-day trial, ORP values as they ranged from $-11$ to +25 mV during the 3 days at a constant effluent nitrate. It should be noted that in trial 1, the ORP probes were not cleaned daily and this likely led to a buildup of organic material which may have resulted in lower than actual ORP values.

In trials 2 and 3 the ORP probes were cleaned daily which mitigated inaccuracies due to organic buildup. In trial 2, Cerelose™, molasses and vinegar fed units reached a constant effluent nitrate at an ORP value of $-227$, $-187$ and $-177$ mV, respectively. Methanol did not reach a constant effluent nitrate until the final 2 days of the trial and demonstrated ORP values of $-20$ to $-150$ mV. In trial 3 Cerelose™, molasses and vinegar fed units reached a constant effluent nitrate at an ORP value of $-235$, $-229$ and +30 mV, respectively. Methanol reached a constant effluent nitrate at day 5 of operation at an ORP value of $-116$ mV.

### 3.4. Nitrate removal

As expected, the g $NO_3$–N removed/m$^3$/h is greatest at the most elevated system nitrate concentrations, and decreases as system concentrations decrease (Fig. 4). All four carbon sources gave essentially the same maximum daily denitrification rate of 0.67–0.68 kg nitrogen removed/m$^3$ media/day. Our calculated rates are in the midrange of the rates reported by other investigators for the same or similar carbon sources (Table 2). All studies referenced in the table focused on waste water treatment with a variety of laboratory and pilot plant systems; no reports of daily nitrogen removal rates in commercial aquaculture systems were found in the literature. This is the first paper to describe the use of molasses as a carbon source for nitrogen removal in a commercial recirculating aquaculture system.

### 3.5. Nitrite formation

Under aerobic conditions, it is energetically more favorable for bacteria to utilize molecular oxygen in the presence of organic electron donors. Under anoxic

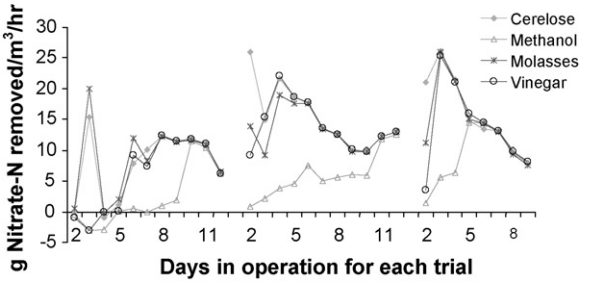


Fig. 4. Gram nitrate–N removed per hour of outlet flows for commercial denitrification units supplemented with four different carbon sources. The units were engaged when the recirculating system was at 55 mg/l $NO_3$–N and disengaged when the system dropped to 10 mg/l $NO_3$–N. This was repeated for three cycles. The filters remained static in between cycles.

Table 2
Comparison of documented denitrification rates (kg/m$^3$/day) using various carbon sources

| Carbon source | Denitrification rate (g $NO_3$–N removed/m$^3$/day) | System | Input $NO_3$–N (mg/l) | Reference |
|---|---|---|---|---|
| Methanol | 670[a] | Freshwater aquaculture | 50 | This study |
| Methanol | 43[b] | Marine aquaculture (eel) | 150 | Suzuki et al., 2003 |
| Methanol | 158[b] | Marine aquaculture (shrimp) | 165 | Menasveta et al. (2001) |
| Methanol | 240–480[c] | Groundwater | 22 | Gomez et al. (2000) |
| Acetic acid | 670[a] | Freshwater aquaculture | 50 | This study |
| Acetic acid | 1300–2000[c] | Tap water | 25 | Aesoy et al. (1998) |
| Acetic acid | 1630[d] | Artificial groundwater | 50 | Kessreu et al. (2002) |
| Hydrolyzed starch | 680[a] | Freshwater aquaculture | 50 | This study |
| Soluble starch | 460 | Groundwater | 13–17 | Kim et al. (2002) |
| Immobilized starch | 624[c] | Freshwater aquarium (goldfish) | 70 | Tal et al. (2003) |
| Immobilized starch | 62[c] | Marine aquarium (cichlids) | 14 | Tal et al. (2003) |
| Sucrose | 240–480[c] | Groundwater | 22 | Gomez et al. (2000) |
| Glucose | 10[b] | Artificial fresh and salt water | 3.5 | Park et al. (2001) |
| Molasses | 670[a] | Freshwater aquaculture | 50 | This study |

[a] Maximum removal rate normalized to 50 mg/l nitrate–N input.
[b] Converted from mg/l/day to g/m$^3$/day.
[c] Pilot plant study.
[d] Laboratory study.

conditions, nitrate becomes the most favorable terminal electron acceptor, releasing one nitrite ion for each nitrate ion, resulting in an undesirable release of nitrite (Gee and Kim, 2004). In the presence of an excess of organic electron donors however, both nitrate and nitrite can be utilized resulting in the production of nitrogen gas which can enter the atmosphere and thereby exit the system. Possible denitrification pathways are shown in the following equations:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO\,(\text{nitric oxide}) \rightarrow N_2O\,(\text{nitrous oxide}) \rightarrow N_2 \quad (10)$$

$$NO_3^- \rightarrow NH_2OH\,(\text{hydroxylamine}) \rightarrow NH_3\,(\text{ammonia}) \rightarrow \text{organic N} \quad (11)$$

Eq. (10) is favorable in terms of removing nitrogen from the system (Brazil, 2004). This pathway is thought to predominate when a relatively narrow range of bacteria can degrade the carbon source (Van Rijn et al., 2006). Methanol and vinegar (acetic acid) are such sources.

It was apparent in this study that prior to the denitrification units reaching a constant effluent nitrate, the resident population of bacteria capable of converting nitrite to nitrogen gas did not generate enough microbial biomass to facilitate the process, and significant concentrations of nitrite accumulated, especially for units fed molasses and Cerelose™ in trial 1, in which nitrite concentrations reached $24.6 \pm 4.1$ and $21.1 \pm 5.6$ mg/l $NO_2$–N, respectively (Fig. 5A). Once at a constant effluent concentration for nitrate, Cerelose™, molasses, vinegar and methanol fed units did not generate nitrite, and in fact nitrite concentrations were often 0.0 mg/l or were significantly reduced in the Cerelose™, molasses and vinegar fed units.

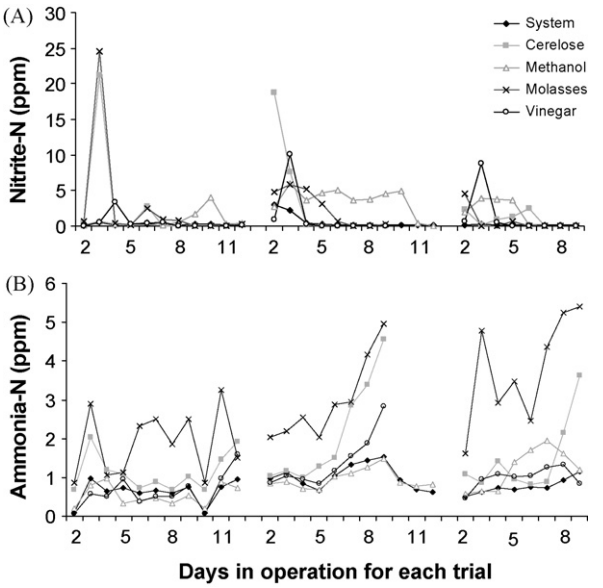


Fig. 5. Nitrite–N (A) and ammonia–N (B) values for inlet (system) and outlet flows for commercial denitrification units supplemented with four different carbon sources. The units were engaged when the recirculating system was at 55 mg/l $NO_3$–N and disengaged when the system dropped to 10 mg/l $NO_3$–N. This was repeated for three cycles. The filters remained static in between cycles.

### 3.6. Ammonia production

Eq. (11) is undesirable since ammonia is highly toxic to most aquatic species (Ackerman et al., 2006; Colt, 2006; Eschar et al., 2006). Both denitrification and fermentative bacteria can utilize an easily degradable carbon source such as molasses or Cerelose™. This reaction can take place under aerobic and anaerobic conditions (Van Rijn et al., 2006). The ammonia can then be assimilated into organic amino groups. It is also possible to produce ammonia by the dissimilatory nitrate reduction to ammonia (DNRA). The process is conducted by fermentative bacteria when the reduction of organic matter is not possible (Tiedje, 1990; Van Rijn et al., 2006). High C/N ratios are thought to favor the DNRA process (Tiedje, 1990).

The ammonia levels in the effluent from the reactors increased during the trials. Maximum measured concentrations in the reactor effluents for methanol, vinegar, Cerelose™ and molasses were $1.62 \pm 0.10$, $2.83 \pm 0.17$, $4.55 \pm 0.45$ and $5.25 \pm 1.26$ mg/l $NH_3$–N, respectively (Fig. 5B). The ammonia concentration in the methanol fed reactor increased at a steady rate whereas the other sources increased more rapidly as the trials neared their end. The reductions of carbon input to the reactors necessarily lagged the drop in nitrogen levels due to the time required for sample analysis. This coupled with the very conservative estimates of the required C/N ratios needed for Cerelose™ and molasses, resulted in C/N ratios higher than needed for complete denitrification. Based on these data, a reasonable hypothesis may follow that for methanol and possibly vinegar the ammonia formed is from the DNRA process, while for the more easily degradable Cerelose™ and molasses, when coupled with high C/N ratios, the assimilative nitrate reduction process dominates. This results in high levels of ammonia and biomass on the media.

### 3.7. Alkalinity and pH

Nitrification leads to an alkalinity loss and a concomitant reduction in pH. Acidic conditions negatively impact microbial performance of the biofilter which can deteriorate water quality. Alkalinity supplements such as sodium bicarbonate are often added to the culture water to remediate reductions. Denitrification reactors result in an alkalinity gain which can ameliorate or reduce the need for supplementation. In trial 1, molasses and vinegar fed units experienced significantly increased alkalinity concentrations once at a constant effluent nitrate (Fig. 6A). Methanol fed units

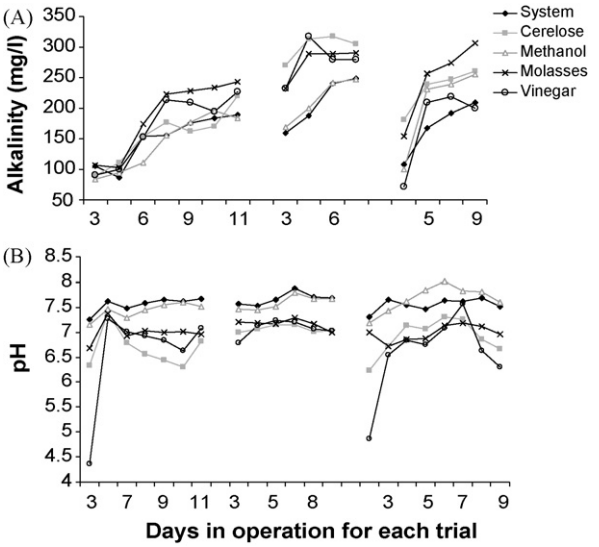


Fig. 6. Alkalinity (A) and pH (B) measurements for inlet (system) and outlet flows for commercial denitrification units supplemented with four different carbon sources. The units were engaged when the recirculating system was at 55 mg/l $NO_3$–N and disengaged when the system dropped to 10 mg/l $NO_3$–N. This was repeated for three cycles. The filters remained static in between cycles.

did not produce significant increases in alkalinity and Cerelose™ did not produce significant increases until day 11 of operation. In trial 2, Cerelose™, molasses and vinegar fed units all experienced significant increases in alkalinity, while methanol fed units did not. Trial 3 was

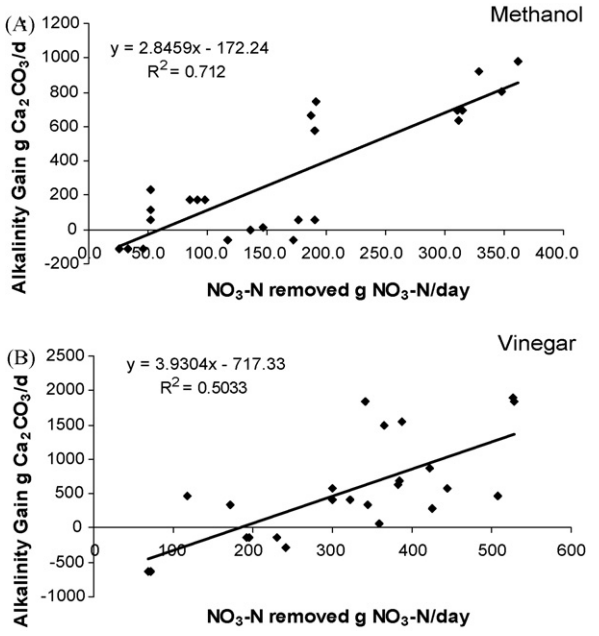


Fig. 7. Alkalinity gains of denitrification units supplemented with either methanol (A) or vinegar (B).

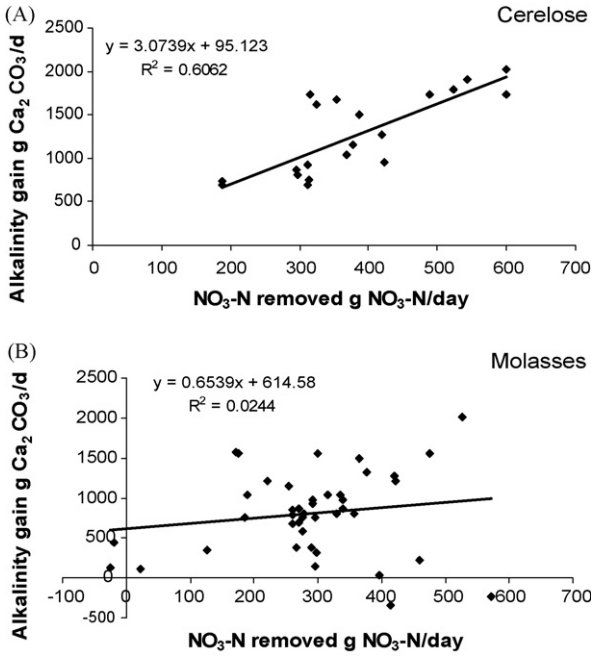


Fig. 8. Alkalinity gains of denitrification units supplemented with either Cerelose™ (A) or molasses (B).

comparable to trial 2, however the vinegar fed units appeared less stable and alkalinity production dropped to insignificant concentrations by day 9 of operation. There was a significant correlation with alkalinity gain and $NO_3$–N reduced for all carbon sources tested except molasses (Figs. 7 and 8).

Interestingly, there was not a concomitant increase in pH as might be expected with increases in alkalinity (Fig. 6B). In fact, other than day 4 of trial 1 for all carbon sources and day 7 for vinegar, the Cerelose™, molasses and vinegar fed units all experienced significant reductions in pH. pH is a function of both alkalinity and acidity concentrations. We can see from Eqs. (1)–(8) that $CO_2$ is produced following degradation of the organic matter. $CO_2$ acidifies the aquatic environment, thereby reducing the pH, and likely accounts for the reductions in pH seen in this study. Methanol fed units did not alter pH concentrations in trials 1 and 2, and experienced a transient increase on days 5 and 6 of trial 3.

### 3.8. Turbidity

Although increased turbidity is not necessarily detrimental to the health and well being of aquatic inhabitants, excess turbidity can be a nuisance in terms of evaluating fish behavior, observing uneaten feed and

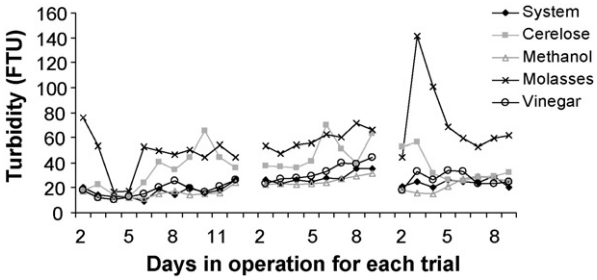


Fig. 9. Turbidity (FTU) values for inlet (system) and outlet flows for commercial denitrification units supplemented with four different carbon sources. The units were engaged when the recirculating system was at 55 mg/l $NO_3$–N and disengaged when the system dropped to 10 mg/l $NO_3$–N. This was repeated for three cycles. The filters remained static in between cycles.

other management concerns. It was clear from this study that molasses led to significant increases in turbidity in all three trials (Fig. 9). Although Cerelose™ fed units produced significantly increased turbidity in trials 1 and 2, by trial 3 these significant increases were no longer present.

### 3.9. COD availability

COD measurements are used to quantify the mass of potential carbon available to fuel the denitrification process. The COD in the outflow of each denitrifying reactor was measured and showed that Cerelose™, molasses and vinegar fed units contained significantly elevated COD concentrations, while methanol fed units contained equivalent COD concentrations to system values (Fig. 10). These data imply that the reactors were not carbon limited, and were receiving enough carbon to facilitate the denitrification process.

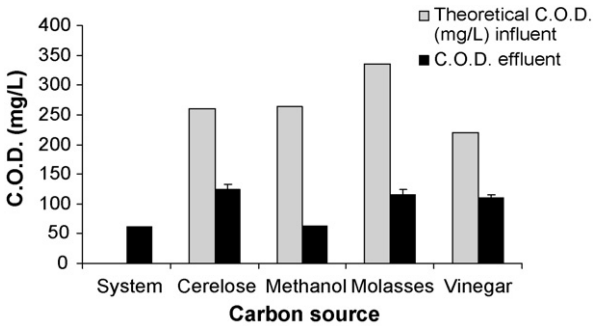


Fig. 10. Chemical oxygen demand (COD) values for theoretical influent and actual outlet flows for commercial denitrification units supplemented with four different carbon sources. COD concentrations were taken on day 7 of trial 2.

### 3.10. Off-flavor

An economically significant problem in aquaculture is ''off-flavor'' in the cultured product. The most common types of off-flavors that have been cited in aquaculture products are ''earthy'' and ''musty'' and these off-flavors are due to the accumulation of geosmin and 2-methylisoborneol, respectively, in the flesh of the cultured organism (Tucker, 2000). Geosmin and MIB are produced by microorganisms such as certain species of actinomycetes, cyanobacteria (blue-green), and fungi (Schrader and Rimando, 2003), and these compounds can be detected by humans at very low concentrations (e.g., less than 10 ng/l) (Ho et al., 2004). While the source(s) of earthy and musty off-flavors in recirculating systems is currently not well understood, some species of actinomycetes are capable of denitrification (Shoun et al., 1998; Kumon et al., 2002), and those species of actinomycetes that are facultative anaerobes may be present in low oxygen or anoxic environments, similar to those present in denitrification environments (e.g., denitrification reactor).

The levels of geosmin and MIB were measured in each reactor to determine the following: (1) if the reactors generated significant quantities of these off-flavor compounds; and (2) if there was differential production of these compounds due to any of the various carbon sources tested. Results revealed that there was no production of either geosmin or MIB for any of the carbon sources tested (Fig. 11). This is a significant finding since the production of off-flavor compounds such as geosmin and MIB would reduce the feasibility of utilizing these units in commercial culture systems in which off-flavor may be a concern.

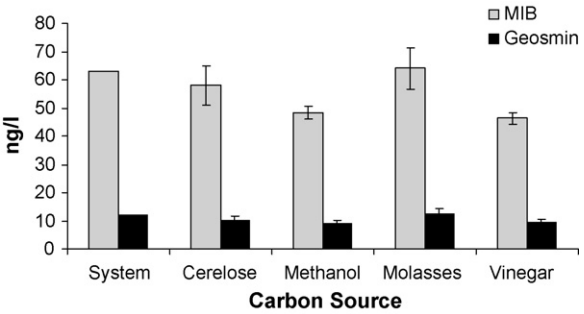


Fig. 11. Off-flavor compound (MIB and geosmin) values for inlet (system) and outlet flows for commercial denitrification units supplemented with four different carbon sources. Samples for off-flavors were taken on day 7 of trial 3.

## 4. Conclusions

The denitrification reactor design used in this study was effective at significantly reducing nitrate concentrations within a relatively short timeframe. ORP values required for the units to reach a constant effluent nitrate were dependant upon the supplemental carbon source, with methanol fed units demonstrating higher ORP values than Cerelose™, molasses or vinegar fed units. Although nitrite production was not a problem in this study once the reactors achieved a constant effluent nitrate, ammonia production was a significant problem for units fed molasses and to a less extent Cerelose™. None of the carbon sources tested enhanced the production of the off-flavor compounds geosmin and MIB, an important consideration for food-fish aquaculture. Because of its very low cost compared to the other sources tested, molasses may be an attractive carbon source for denitrification if issues of ammonia production, turbidity and foaming can be resolved. Based on our results from these trials, much lower C:N ratios should be possible. Additional studies of molasses as a carbon source are needed.

## Acknowledgements

This research was cooperatively funded by the Southwest Florida Water Management District/Manasota Basin Board and Mote Scientific Foundation. We would like to thank Brian E. Babbitt and Brian A. Richard II for construction of the denitrification reactors and Travis Smith, Wesley Ripperger and Randy Shine for technical assistance and maintenance throughout the project. The technical assistance of Ramona Pace (USDA, ARS, NPURU) is also greatly appreciated.

## References

Ackerman, P.A., Wicks, B.J., Iwama, G.K., Randall, D.J., 2006. Low levels of environmental ammonia increase susceptibility to disease in Chinook salmon smolts. Physiol. Biochem. Zool. 79, 695–707.

Aesoy, A., Odegaard, H., Back, K., Rujol, R., Harmon, M., 1998. Denitrification in a packed biofilm reactor (BIOFOR)—experiments with different carbon sources. Water Res. 32, 1463–1470.

Balderston, W., Sieburth, J.M., 1976. Nitrate removal in closed-system aquaculture by columnar denitrification. Appl. Environ. Microbiol. 32, 808–818.

Boyd, C.E., Tucker, C.S., 1998. Sustainability and Environmental Issues. Pond Aquaculture and Water Quality Management, pp. 601–624.

Breck, W.G., 1974. Redox levels in the sea. In: Goldberg, D. (Ed.), The Sea—Ideas and Observations on Progress in the Study of the Seas, Marine Chemistry, vol. 15. Wiley, New York, pp. 153–180.

Buschmann, A.H., Lopez, D.A., Medina, A., 1996. A review of the environmental effects and alternative production strategies of a marine aquaculture in Chile. Aquacult. Eng. 34, 163–171.

Chang, C.N., Cheng, H.B., Chao, A.C., 2004. Applying the Nernst equation to simulate redox potential variations for biological nitrification and denitrification processes. Environ. Sci. Technol. 38, 1807–1812.

Colt, J., 2006. Water quality requirements for reuse systems. Aquacult. Eng. 34, 143–156.

Cranford, P., Dowd, M., Grant, J., Hargrave, B., McGladdery, S., 2003. Ecosystem level effects of marine bivalve aquaculture In: A scientific review of the potential environmental effects of aquaculture in aquatic ecosystems. Vol. I. Fisheries and Oceans Canada. Can. Tech. Rep. Fish. Aquat. Sci. 2450, 12–20.

Cuervo-Lopez, F.M., Martinez, L., Gutierrez-Rojas, M., Noyola, R.A., Gomez, J., 1999. Effect of nitrogen loading rate and carbon source on denitrification and sludge settleability in upflow anaerobic sludge blanket (UASB) reactors. Water Sci. Tech. 40, 123–130.

Eschar, M., Lahav, O., Mozes, N., Peduel, A., Ron, B., 2006. Intensive fish culture at high ammonia and low pH. Aquaculture 255, 301–313.

FAO, 1995. Code of conduct for Responsible Fisheries. Food and Agriculture Organization of the United Nations, Rome, 41 pp.

Frankic, A., Hershner, C., 2003. Sustainable aquaculture: developing the promise of aquaculture. Aquacult. Int. 11, 517–530.

Gee, C.S., Kim, J.S., 2004. Nitrite accumulation followed by denitrification using sequencing batch reactor. Water Sci. Tech. 49, 47–55.

Gomez, M., Gonzalez-Lopez, J., Honotia-Garcia, E., 2000. Influence of carbon source on nitrate removal of contaminated groundwater in a dentrifying submerged filter. J. Hazard. Mater. B80, 69–80.

Greenberg, A.E., Clesceri, L.S., Eaton, A.D., 1992. Standard Methods for the Examination of Water and Wastewater. APHA, Washington.

Grguric, G., Wetmore, S.S., Fournier, R.W., 2000. Biological denitrification in a closed seawater system. Chemosphere 40, 549–555.

Grommen, R., Verhaege, M., Verstraete, W., 2006. Removal of nitrate in aquaria by means of electrochemically generated hydrogen gas as electron donor for biological denitrification. Aquacult. Eng. 34, 33–39.

Guillette Jr., L.J., Edwards, T.M., 2005. Is nitrate an ecologically relevant endocrine disruptor in vertebrates? Integr Comp. Biol. 45, 19–27.

Gutierrez-Wing, M.T., Malone, R.F., 2006. Biological filters in aquaculture: trends and research directions for freshwater and marine applications. Aquacult. Eng. 34, 163–171.

Hall, A.G., Hallerman, E.M., Libey, G.S., 2002. Comparative analysis of performance of three biofilter designs in recirculating aquaculture systems. In: Proceedings of the 4th International Conference on Recirculating Aquaculture.

Hamlin, H.J., 2006. Nitrate toxicity in Siberian sturgeon (*Acipenser baeri*). Aquaculture 253, 688–693.

Harache, Y., 2002. Responsible aquaculture in the next century: an evolutionary perspective. In: Creswell, R.L., Flos, R. (Eds.), Perspectives on Responsible Aquaculture for the New Millenium. World Aquaculture Society/The European Aquaculture Society, Baton Rouge, LA, USA/Oostende, Belgium, pp. 1–27.

Ho, L., Croué, J.P., Newcombe, G., 2004. The effect of water quality and NOM character on the ozonation of MIB and geosmin. Water Sci. Tech. 49, 249–255.

Houte, A., 2000. Establishing legal, institutional and regulatory framework for aquaculture development and management. In: Conference on Aquaculture in the Third Millennium, Bangkok (Thailand), pp. 103–120.

Hrubec, T.C., 1996. Nitrate toxicity: a potential problem of recirculating systems. Aquacult. Eng. Soc. Proc. 41–48.

Jensen, F.B., 1996. Uptake, elimination and effects of nitrite and nitrate in freshwater crayfish. Aquat. Toxicol. 34, 95–104.

Johnston, D.W., Cross, T., 1976. Actinomycetes in lake muds: dormant spores or metabolically active mycelium? Freshwater Biol. 6, 465–470.

Kessreu, P., Kiss, I., Bihari, Z., Polyak, B., 2002. Investigation of dentrification activity of immobilized Pseudomonas butanovora cells in the presence of different organic substrates. Water Res. 36, 1565–1571.

Kessreu, P., Kiss, I., Bihari, Z., Polyak, B., 2003. Biological denitrification in a continuous-flow bioreactor containing immobilized *Pseudomonas butanovora* cells. Bioresour. Tech. 87, 75–80.

Kim, Y.H., Hensley, R., 1997. Effective control of chlorination and dechlorination at wastewater treatment plants using redox potential. Water Environ. Res. 69, 1008–1014.

Kim, E.W., Bae, J.H., 2000. Alkalinity requirements and the possibility of simultaneous heterotrophic denitrification during sulfur utilizing autotrophic denitrification. Water Sci. Tech. 42, 233–238.

Kim, Y.K., Nakano, K., Lee, T.L., Kanchanatawee, S., Matsumura, M., 2002. On-site nitrate removal of groundwater by an immobilized phychrophilic denitrifier using soluble starch as a carbon source. J. Biosci. Bioeng. 93, 303–308.

Klas, S., Mozes, N., Lahav, O., 2006. A conceptual, stoichiometry-based model for single-sludge denitrification in recirculation aquaculture systems. Aquaculture 259, 328–341.

Kumon, Y., Sasaki, Y., Kato, I., Takaya, N., Shoun, H., Beppu, T., 2002. Codenitrification and denitrification are dual metabolic pathways through which dinitrogen evolves from nitrate in *Streptomyces antibioticus*. J. Bacteriol. 184, 2963–2968.

Lee, P.G., Lea, R.N., Dohmann, E., Prebilsky, W., Turk, P.E., Ying, H., Whitson, J.L., 2000. Denitrification in aquaculture systems: an example of a fuzzy logic control problem. Aquacult. Eng. 23, 37–59.

Lloyd, S.W., Lea, J.M., Zimba, P.V., Grimm, C.C., 1998. Rapid analysis of geosmin and 2-methylisoborneol in water using solid phase micro extraction procedures. Water Res. 32, 2140–2146.

Losordo, T.M., Westeman, P.W., 1994. An analysis of biological, economic, and engineering factors affecting the cost of fish production in recirculation aquaculture systems. J. World Aquacult. Soc. 24, 193–203.

McCarty, P.M., Beck, L., Amant, St.P., 1969. Biological denitrification of wastewaters by addition of organic materials. In: Proceedings of the 24th Industrial Water Conference, Purdue University, pp. 1271–1285.

Menasveta, P., Panritdam, T., Sihanonth, P., Powtongsook, S., Chuntapa, B., Lee, P., 2001. Design and function of a closed, recirculating seawater system with denitrification for the culture of black tiger shrimp broodstock. Aquacult. Eng. 25, 35–49.

Mohseni-Bandpi, A., Elliot, D., Momeny-Mazdeh, A., 1999. Denitrification of ground water using acetic acid as a carbon source. Water Sci. Tech. 40, 53–59.

Olin, P.G., 2001. Current status of aquaculture in North America. In: Conference on Aquaculture in the Third Millenium, Bangkok (Thailand), pp. 377–396.

Park, E.J., Seo, J.K., Kim, M.R., Jung, I.H., Kim, J.Y., Kim, S.K., 2001. Salinity acclimation of immobilized freshwater denitrifiers. Aquacult. Eng. 24, 169–180.

Pita, P., Apostolaki, E.T., Tsagaraki, T., Tsapakis, M., Karakassis, I., 2006. Fish farming effects on chemical and microbial variables of the water column: a spatio-temporal study along the Mediterranean Sea. Hydrobiologia 563, 99–108.

Russo, R.C., 1985. Ammonia, nitrite, and nitrate. In: Rand, G.M., Petrocelli, S.R. (Eds.), Fundamentals of Aquatic toxicology Methods and Applications. Hemisphere Publishing, pp. 455–471.

Schrader, K.K., Rimando, A.M., 2003. Off-flavors in aquaculture: an overview. In: Rimando, A.M., Schrader, K.K. (Eds.), Off-Flavors in Aquaculture ACS Symposium Series, vol. 848. American Chemical Society, Washington, DC, pp. 1–12.

Schrader, K.K., Nanayakkara, N.P.D., Tucker, C.S., Rimando, A.M., Ganzera, M., Schaneberg, B.T., 2003. Novel derivatives of 9, 10-anthraquinone are selective algicides against the musty-odor cyanobacterium Oscillatoria perornata. Appl. Environ. Microbiol. 69, 5319–5327.

Sillen, L.G., 1965. Oxidation states of Earth's ocean and atmosphere: a model calculation on earlier states: the myth of prebiotic soup. Archiv. Kemi. Acta 24, 431–456.

Sharma, B., Ahlert, R.C., 1977. Nitrification and nitrogen removal. Water Res. 11, 897–925.

Sharrer, M.J., Tal, Y., Ferreir, D., Hankins, J.A., Summerfelt, S.T., 2007. Membrane biological reactor treatment of a saline backwash flow from a recirculating aquaculture system. Aquacult. Eng. 36, 159–176.

Shoun, H., Kano, M., Baba, I., Takaya, N., Matsuo, M., 1998. Denitrification by actinomycetes and purification of dissimilatory nitrite reductase and azurin from *Streptomyces thioluteus*. J. Bacteriol. 180, 4413–4415.

Sperl, G.T., Hoare, D.S., 1971. Denitrification with methanol: selective enrichment for *Hyphomicrobium* spp. J. Bacteriol. 108, 733–736.

Spotte, S., 1979. Seawater Aquariums: The Captive Environment. Wiley, New York.

Suzuki, Y., Maruyama, T., Numata, H., Sato, H., Asakawa, M., 2003. Perormance of a closed recirculating system with foam separation, nitrification and denitrification units for intensive culture of eel: towards zero emission. Aquacult. Eng. 29, 165–182.

Tal, Y., Nussinovitch, A., van Rijn, J., 2003. Nitrate removal in aquariums by immobilized denitrifers. Biotechnol. Prog. 19, 1019–1021.

Tiedje, J.M., 1990. Ecology of denitrification and dissimilatory nitrate reduction to ammonia. In: Zehnder, A.J.B. (Ed.), Biology of Anaerobic Microorganisms. Wiley, NY, pp. 179–244.

Timmons, M.B., Ebeling, J.M., Wheaton, F.W., Summerfelt, S.T., Vinci, B.J., 2001. Recirculating Aquaculture Systems. NRAC Publication no. 01-002. Cayuga Aqua Ventures, Ithaca, NY, 650 pp.

Tucker, C.S., 2000. Off-flavor problems in aquaculture. Rev. Fish. Sci. 8, 45–88.

Van Rijn, J., 1996. The potential for integrated biological treatment systems in recirculating fish culture—a review. Aquaculture 139, 181–201.

Van Rijn, J., Tal, Y., Schreier, H.J., 2006. Denitrification in recirculating systems: theory and applications. Aquacult. Eng. 34, 364–376.